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Quantitative genetic manipulation for nauplii size reduction of *Artemia franciscana* Kellogg, 1906 from Indian salinas and correlated changes in the polyunsaturated fatty acids (PUFA) profile

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ABSTRACT

Thirteen generations of mass selection was carried out in a strain of *Artemia franciscana* collected from an Indian salina. The primary trait under selection was nauplii length, and the criteria of selection was small size. While 12.4% reduction (from 517.0±59.8 µm to 452.2±25.0 µm) was realised in the trait under selection from 13 generations, substantial increase in the polyunsaturated fatty acids (PUFA) content was realised as correlated response. The polyunsaturated fatty acid (PUFA) content showed a steady increase during the selection. The PUFA percentage in G2, G4, G6, G9, G11 and G13 generations were 21.43, 27.96, 27.19, 33.27, 36.98 and 37.25 respectively compared to 18.04 in the base generation. The content of essential polyunsaturated fatty acids such as 20:5n-3 and 22:6n-3 were also high in the selected generations, compared to the base generation indicating their nutritional superiority. The smaller nauplii with an enhanced level of PUFA, especially the essential polyunsaturated fatty acids, developed through selective breeding in the present work make it a promising strain as live feed for larviculture of marine species.

Keywords: *Artemia*, Correlated change, Naupliar size, PUFA, Selective breeding

Introduction

The brine shrimp *Artemia* is a hyper saline crustacean, distributed world wide in the inland salt lakes, solar salt works and coastal lagoons of the three major temperature regions *viz.*, tropical, subtropical and temperate regions (Van Stappen *et al*., 2001; Eimanifar *et al*., 2006). *Artemia* comprise bisexual species and a number of parthenogenetic populations with a variety of ploidies as di-, tri-, tetra-, penta- and heteroploid groups under the binomen *Artemia parthenogenetica* (Sun *et al*., 1999). *Artemia* have the ability to produce embryos or cysts or give birth to nauplii in large quantities. In finfish and shellfish hatcheries, *Artemia* nauplii are extensively used as live feed (Treece, 2000). Nauplii size is the most important factor in larviculture. This is because most of the finfish larvae are small with narrow buccal openings, and therefore, exogenous foods of smaller size are needed for better survival. In general *Artemia* nauplii are larger (500-550 µm) and through selective breeding *Artemia* strains can be developed with smaller naupliar size (Shirdhankar and Thomas, 2003).

High level of polyunsaturated fatty acids (PUFA) is as vital as small naupliar size in larviculture. Due to the high growth rate in the early developmental phase of the larvae, the starter diet should hold high levels of fatty acids, especially the *n*-3 and *n*-6 PUFAs. It is well known that in finfish and shellfish larvae, these PUFAs play major role in many physiological processes (Clegg and Trotman, 2002; Dantagnan *et al.,* 2010). They have vital roles in membrane structure and function and also serve as important energy sources during the early development of the larvae (Tocher, 2003). Hence, the combined effect on higher PUFA levels and nauplii size reduction in *Artemia franciscana* by selective breeding was evaluated in this work.

Materials and methods

Collection of indigenous Artemia

Following the exploratory survey in the hypersaline water bodies and salterns of Indian sub-continent, cysts of indigenous *Artemia* from Kelambakkam in Tamil Nadu were collected in the month of February, 2008. They were hatched out following the standard procedure (Vanhaecke and Sorgeloos, 1980) and the nauplii were maintained in the hatchery in cylindrical acrylic tanks of 10 l capacity containing 35‰ seawater with pH 7.5. Optimum temperature of 25±1^{0}C with mild

aeration and photoperiod of 12 h D: 12 h L were provided in all the tanks. This formed the base generation (G_0). Microalgae *Isochrysis galbana* was given as the live feed (30 X 10^4 cells ml^{-1}). Morphometric traits like first day length (FDL), third day length (TDL) and sixth day length (SDL) were measured, their mean values and standard deviation were also estimated. Morphological observations and breeding trials conducted (Vikas *et al*., 2012) revealed that they are sexually breeding, and molecular analysis using ITS-1 sequence confirmed that they belong to the species *Artemia franciscana*.

Selective breeding

Base generation (G_0) of the indigenous *A. franciscana* from Kelambakkam were subjected to selective breeding for nauplii size reduction. They were pair mated and the pedigree hatched nauplii were used for selection. Nauplii length/first day length (FDL) was recorded using a microscope attached with DIGI EYE 330 camera and software (Dewinter Biowizard, India), and the nauplii with small size were selected (approximately 10,000) using mesh filter of 500 µm and used to produce the first selected generation (G_1). Nauplii were grown for 10 days in individual containers of 10 l capacity containing seawater (salinity 35±2‰) under standard culture conditions described above. The third day length (TDL) and sixth day length (SDL) were also measured. Based on the FDL of nauplii, mass selection was practiced for 13 generations wherein small nauplii (approximately 10,000 from each generation) were selected using mesh filter of 500 µm. Data on individual progenies were recorded family-wise in every generation, and analysed to estimate the selection response and correlated response. Representative samples were preserved from every selected generation as well as from the base generation for analyses of the fatty acid composition. Selection differential in the base generation was -25.97 and it showed variations over the generations. It ranged from -33.17 to -8.66 µm in other generations. Phenotypic standard deviation, selection differentials and standard selection differential of the generations are presented in Table 1.

Estimation of fatty acid composition

Since the correlated changes in the fatty acid profile of nauplii following selective breeding for nauplii size reduction was intended to be worked out, the fatty acid profile of the base generation and the selected generations were estimated. Lipid content was estimated following Bligh and Dyer (1959) with suitable modifications. In brief, the samples (500 mg) were extracted with $CHCl_3$/MeOH (60 ml, 2:1, v/v) and water (20 ml). The $CHCl_3$ layer was processed to recover the triglycerides which were saponified with alkaline reagent (3 ml, 0.5 N KOH/MeOH). The saponified materials were thereafter reacted with a methylating mixture (14% BF_3/CH_3OH) to yield methyl esters (FAME) that was subsequently extracted with *n*-hexane/H_2O (1:2, v/v) (Metcalf *et al.,* 1966). After removal of the aqueous layer, the *n*-hexane layer was passed through Na_2SO_4, concentrated *in vacuo*, reconstituted in petroleum ether, and stored at -20°C until required for analyses. A Perkin Elmer Auto System XL, Gas chromatograph (Perkin Elmer, USA) equipped with a flame ionization detector (FID) analysed the composition of the fatty acids. The esterified fatty acid content of the *Artemia* cyst, nauplii and adult were analysed by gas liquid chromatography with comparison to fatty acid methyl ester standard (Supelco FAME 37 standard).

Table 1. Selection differential (µm), phenotypic standard deviation (µm) and standardised selection differential of the different generations of *Artemia franciscana*

Generation	Selection differential (µm)	Phenotypic standard deviation (µm)	Standard selection differential
G0	-25.97	39.84	-0.65
G1	-33.17	35.52	-0.93
G2	-29.98	38.59	-0.78
G3	-32.54	34.38	-0.95
G4	-24.17	38.75	-0.62
G5	-25.78	27.48	-0.94
G6	-21.26	23.15	-0.92
G7	-16.52	27.19	-0.61
G8	-13.73	27.10	-0.51
G9	-10.19	30.13	-0.34
G10	-11.43	24.86	-0.46
G11	-11.82	21.44	-0.55
G12	-14.19	29.19	-0.49
G13	NA	25.09	NA

Results and discussion

Response to selection

Substantial reduction in nauplii size (first day length-FDL) was achieved as direct response to the selective breeding for small size nauplii. The FDL could be reduced from 517.0 µm to 452.2 µm during the 13 generations of selective breeding. Generation-wise mean values with standard deviation of nauplii length are presented in Table 2. A gradual decrease in nauplii length was noticed during the selection process. Cumulative selection gain during the thirteen generations of selection was -64.78 µm. Correlated response in other biometric traits such as third day length (TDL) and sixth day length (SDL) are also presented in Table 2.

Correlated response in fatty acid content

Correlated changes in the fatty acid content were obvious in different selected generations of *Artemia* (Table 3). The total PUFA steadily increased during the selection (37.25%) when compared to base generation (18.04%). The fatty acids, 18:2n6, 18:3n6, 18:3n3, 20:2n6 and 20:5n3 contributed the major share of the total PUFA in the selected *Artemia.*

Majority of PUFAs belonged to the omega-3 and omega-6 type, and ranged between C18 to C22 in chain length. Eicosapentaenoic acid (EPA, 20:5n3) which appeared to be low in initial generations showed a sharp increase by 13th generation. The docosahexaenoic acid (DHA, 22:6n3) also increased after the selection and reached high levels by 13th generation. Similarly, arachidonic acid (20:4n6) also increased substantially. Total n6 fatty acids increased during the selection (G13-20.48%), as compared to the base (G0) generation (11.33%). Similarly, the total n3 fatty acids showed substantial increase in the selected generations *viz.,* 16.77% in the thirteenth generation as against 6.71% in the base (G0) generation. The n3/n6 ratio was low (<1) in the selected generations (0.59 to 0.82) except in G2 and G4. The total PUFA/total SFA ratio was high from the 9th generation onwards (1.24 to 1.33). DHA/EPA ratio in the selected generations ranged from 0.10 to 0.87, except in G4. Though, the unsaturated and mono-unsaturated fatty acid levels decreased consequent to the nauplii size reduction, there was a substantial increase in the PUFA content, which is the most valuable fraction of fatty acid from the nutritional point of marine larval feeding.

The present investigation revealed the change in body size and the PUFA level of *A. franciscana* during selective breeding (Fig. 1), which was inversely correlated.

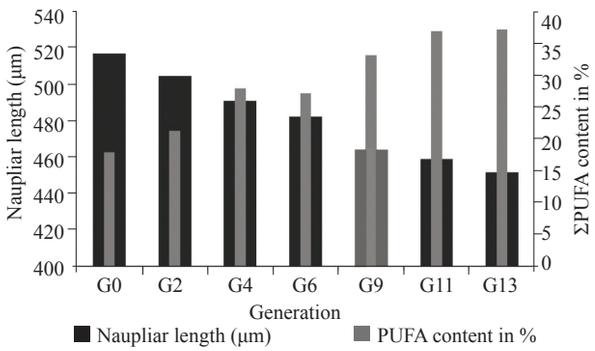

Fig. 1. Correlated response of selective breeding in the naupliar length and PUFA content of *Artemia franciscana*

Table 2. Response of selection in body size at different stages in the different generations of *Artemia franciscana*

Generation	Selection differential (µm)	Direct response		Correlated response	
		First day length (FDL, µm)	Cumulative gain (µm)	Third day length (TDL, mm)	Sixth day length (SDL, mm)
G0	-25.97	517.0±59.8	NA	1.28 ± 0.19	3.67 ± 0.89
G1	-33.17	514.6±20.5	-2.40	1.29 ± 0.20	3.65 ± 0.84
G2	-29.98	504.7±38.5	-12.26	1.29 ± 0.21	2.63 ± 0.56
G3	-32.54	501.7±20.3	-15.23	1.05 ± 0.21	2.53 ± 0.48
G4	-24.17	491.0±38.7	-25.93	1.05 ± 0.17	2.58 ± 1.10
G5	-25.78	490.1±19.4	-26.89	1.20 ± 0.22	3.07 ± 0.30
G6	-21.26	482.5±23.1	-34.49	1.06 ±0 .11	2.25 ± 0.40
G7	-16.52	477.1±27.1	-39.82	1.44 ± 0.28	2.73 ± 0.44
G8	-13.73	471.4±27.1	-45.60	1.15 ± 0.05	2.82 ± 0.24
G9	-10.19	464.1±30.1	-52.81	1.15 ± 0.07	3.08 ± 0.82
G10	-11.43	463.4±24.8	-53.57	1.26 ± 0.06	2.39 ± 0.28
G11	-11.82	459.4±21.4	-57.51	1.25 ± 0.24	3.12 ± 0.32
G12	-14.19	454.5±29.1	-62.47	1.13 ± 0.03	2.58 ± 0.34
G13	NA	452.2±25.0	-64.78	1.14 ± 0.06	2.20 ± 0.24

Table 3. Fatty acid content in different selected generations of *Artemia franciscana*

Fatty acids (%TFA)	Generation number						
	Base	Gen. 2	Gen. 4	Gen. 6	Gen. 9	Gen. 11	Gen. 13
Saturated fatty acids	31.11	41.90	35.08	33.42	29.97	29.83	30.02
Mono-unsaturated fatty acids	42.39	32.91	28.20	28.99	28.50	25.44	25.90
PUFAs							
18:2n6	7.52	2.17	3.91	3.98	5.67	2.58	4.13
18:3n6	1.18	1.55	4.79	5.76	9.36	8.20	6.58
18:4n6	0.03	0.62	0.64	0.59	0.37	0.41	0.26
18:2n3	0.00	0.00	0.56	0.57	0.96	0.59	0.62
18:3n3	3.21	3.93	5.59	5.48	5.59	5.40	4.05
18:4ω3	0.08	3.35	0.56	0.59	0.66	0.72	5.15
20:2n6	0.15	1.65	0.32	1.10	1.40	4.84	5.74
20:3n6	1.10	0.88	0.08	0.98	1.18	0.88	0.74
20:4n6	1.35	1.70	1.20	1.60	1.90	3.70	3.03
20:5n3	3.08	2.88	2.96	3.10	3.60	5.11	3.59
22:5n3	0.02	0.36	1.76	1.26	1.11	0.06	0.22
22:6n3	1.32	2.34	5.59	2.18	1.47	4.49	3.14
Total PUFA	18.04	21.43	27.96	27.19	33.27	36.98	37.25
∑ n3	6.71	12.86	17.02	13.18	13.39	16.37	16.77
∑ n6	11.33	8.57	10.94	14.01	19.88	20.61	20.48
n3/n6	0.59	1.50	1.56	0.94	0.67	0.79	0.82
∑ PUFA/∑ SFA	0.58	0.51	0.80	0.89	1.33	1.24	1.24
EPA/AA	2.28	1.69	2.47	1.94	1.89	1.38	1.18
DHA/EPA	0.10	0.81	1.89	0.70	0.41	0.88	0.87

Both are very advantageous, since small sized nauplii with high level of PUFA is very much desired for use as live larval feed. It is possible that, like many other traits reported across the species, there might be a negative genetic correlation between body length and PUFA biosynthesis. This forms the first report on the genetic improvement of indigenous strain of *Artemia* through selective breeding.

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